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Microbial diversity and dynamicity of biogas reactors fed with different substrates

Davide De Francisci[1], Panagiotis G. Kougias[1], Laura Treu[1], Stefano Campanaro[2], Irini Angelidaki[1]
[1]Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark
[2]Department of Biology, University of Padua, Via U. Bassi 58/b 35131 Padova, Italy

Anaerobic digestion (AD) is widely applied method for treating different types of wastes. Nowadays, the biogas plants have proliferated and therefore extra biomass is needed in order to meet the surplus needs. In Denmark, most of the biogas plants are co-digesting manure (at an amount of 70%) and other organic residues mainly derived from food industries (at an amount of 30%) (Angelidaki and Ellegaard, 2003). As a consequence, the mixed feedstock is very complex and contains several compounds that could either result in a successful combination for the biomethanation or in contrary could inhibit the AD process. Aim of this study is to gain a deeper understanding on how the microbial ecology of the biogas reactors, mainly constituted by Archaea and Bacteria, responds to radical changes of substrate.

The experimental work was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3, operating under thermophilic conditions (54±1^{o}C). The hydraulic retention time (HRT) of the reactors was kept to 15 days. During the start-up period all the reactors were inoculated with thermophilic inoculum obtained from Hashøj biogas plant, Denmark. The whole experiment was divided in two periods; during the first experimental period all the reactors were fed with cattle manure, while in the second period the substrate contained cattle manure supplemented with proteins (R1), lipids (R2) and carbohydrates (R3). The amount of the different compounds added to the manure was based according to preliminary experiment in order to maintain the level of organic loading rate (OLR) stable (Kougias et al., 2013). Samples were taken from each reactor prior to the feedstock change and after a time period of one HRT during which the reactors were fed with the corresponding mixed substrate. DNA was extracted from each sample and the corresponding microbial composition was determined via analyses of 16S amplicons. During the experiment the daily biogas production, the composition of methane in biogas, pH and the concentration of volatile fatty acids (VFA) were recorded.

Before the substrate change, the microbial composition of the three reactors was found to be extremely similar. The most abundant within the identified genera (excluding < 1% relative abundance) were found to be *Methanobrevibacter*, *Megamonas*, *Flectobacillus*, *Bacteroides*, *Clostridium*, *Myroides*, *Flavobacterium* and *Bacillus*.

After the substrate composition was modified, taking in consideration only the genera whose relative abundance reached at least 1% before or after the change, the profile of the microbial communities substantially changed within a relatively short period of time, corresponding to one HRT.

In the reactor supplemented with carbohydrates, the relative abundance of microorganisms belonging to the genera *Methanobravibacter* and *Bacteroides* show the highest decrease (12 and 9 folds, respectively), *Myroides* 3 folds and *Flavobacterium* 2 folds. These decrements are compensated by the enormous increase (69 folds) of *Lactobacillus* and the more moderate of *Bacillus* (2 folds). In the reactor supplemented with lipids *Methanobrevibacter* and *Bacteroides* are again found to decrease of several folds (6 and 4, respectively) together with *Myroides* (14 folds) *Flectobacillus* (2 folds) and *Bacillus* (decrease of 2 folds). *Megamonas* is the only genus that increased its relative abundance upon the addition of lipids (increase of 2 folds). In contrast with the aforementioned reactors, the one supplemented with proteins did not undergo substantial changes in

its microbial composition. *Methanobrevibacter* is the only genus whose relative abundance varies significantly (decrease of 2 folds).
This simple analysis underlines the dynamicity of microbial communities populating biogas reactors showing that by changing the composition of the main substrate the profile of the microbial community undergoes a profound transformation and is significantly altered already after a relatively short period of time corresponding to one HRT.

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